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# Influence of L-galactonic acid γ-lactone on ascorbate production in some yeasts

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**Abstract**

L-galactonic acid γ-lactone appear to influence ascorbic acid production in strains of *Saccharomyces cerevisiae, Clavispora lusitaniae, Cryptococcus terreus, Pichia fermentans* in which this is undetected whenever glucose represents the sole carbon source. *Cryptococcus terreus* (strains DBVP 6012 and 6242) does not show ascorbic acid production either in presence or in the absence of L-galactonic acid γ-lactone. This feature is probably connected to the insensibility of the strain to the lycorine, an alkaloid which commonly inhibits cell division probably by blocking L-galactonic acid γ-lactone convertion into ascorbate.

## Introduction

Ascorbic Acid (AA) is widely distributed in nature; it has been detected in animals and plants, as well as in microorganisms. In plants and animals the physiological role of ascorbate, in many redox processes, has been thoroughly investigated (Chayen 1953; Loewus 1980; Arrigoni 1994). On the other hand, studies on ascorbate as product of metabolism in microorganisms, although not preliminary, are still inadequate.

The occurrence of ascorbate in *Lipomyces starkeyi* was investigated by Heick et al. (1969). Later on, Heick et al. (1972) studied the apparent AA produced by several yeast strains grown on media containing glucose as carbon source; they were able to select 16 yeast strains out of 42 which resulted negative for the presence of AA, both in the cells and in the culture media.

Bleeg (1966) tested two strains of *Saccharomyces*, in which the biosynthesis of AA occurs when grown on media with glucose as carbon source; the metabolite was mostly found in the cells, and its synthesis was enhanced by adding L-galactonic acid γ-lactone. Nishikimi et al. (1978, 1980) purified, from cells of *S. cerevisiae*, L-galactono-γ-lactone oxidase resembling, in redox property, that of gulono-γ-lactone oxidase, the isofunctional enzyme in animals. Successively, Bleeg & Christensen (1982) purified from the same species a L-galactono-γ-lactone oxidase with properties differing from the ones described by the previous authors.

On the basis of the above mentioned papers, it could be assumed that the addition of L-galactonic acid γ-lactone to the medium could stimulate the production of AA in yeast strains in which its biosynthesis is undetected whenever glucose represents the sole carbon source. In order to verify this stimulation, if occurring, we selected yeast strains on the basis of their uncapability to produce AA when grown on glucose as sole carbon source, i.e. three different strains of *Saccharomyces cerevisiae* Meyen ex Hansen, one of *Clavispora lusitaniae* Rodrigues de Miranda, one of *Pichia fermentans* Lodder, and two of *Cryptococcus terreus* di Menna.

It should be noted that five strains out of seven selected are inhibited in their growth by lycorine (Garuccio & Arrigoni 1989), while the two strains of *C. terreus* are not sensitive to it (Garuccio & Arrigoni 1989; Garuccio et al. 1989a). As previously reported this plant metabolite is capable to inhibit cell divi-

sion (De Leo et al. 1973), probably by blocking L-galactonic acid γ-lactone convertion into ascorbate (De Gara et al. 1994). These evidences could suggest some connections between the production of ascorbate induced by L-galactonic acid γ-lactone and lycorine effects.

Besides exploring the metabolic aspects of ascorbic acid in microorganisms, these studies cold be useful in identifying valid experimental models – e.g. for cytochemical localization of AA –, selecting yeast strains able to enhance AA production only by varying the composition of the culture media.

## Materials and methods

Two strains of *C. terreus* (DBVPG 6012 = CBS 1895 and DBVPG 6242 = CBS 6293), three different strains of *S. cerevisiae* (DBVPG 6174 = CBS 400, DBVPG 6223 = CBS 5112 and DBVPG 6248 = CBS 2354), one of *C. lusitaniae* (DBVPG 6142 = CBS 4413), one of *P. fermentans* (DBVPG 3808), obtained from DBVPG Industrial Yeast Collection (Perugia, Italy), were utilized.

Stock cultures were maintained on Malt Agar (DIFCO) slants, stored at 4–5 °C.

Control cultures were grown on 200 ml liquid Yeast Nitrogen Base (YNB, DIFCO), containing 0.5% glucose, in 250 ml Erlenmeyer flasks, which were continuously shaken (100 strokes per minute) at 25 °C for 5 days in darkness. Treated cultures were grown on the same medium containing, in addition, 5 mM L-galactonic acid γ-lactone. Yeast cells were collected by filtrating culture broths through Millipore cellulose acetate membrane (pore-size 0.45 μm). Cell homogenates were prepared by grinding, in the dark, the biomass in a precooled (–20 °C) mortar: wet cells (1 g) were suspended in 5% metaphosphoric acid (10 ml) and ground in the presence of siliceous sand (9 g), providing the temperature did not rise above 0° C during the grinding. The extent of cell breakage reached the value of 70%, as verified by microscopic observation. The average grinding time was 20–30 min. The sand and the cell-débris were subsequently removed by centrifugation at 5 °C, for 20 min at 16,000 × g. The AA was determined by monitoring the decrease of absorbance at 265 nm, at 30 °C, at pH 6.4, according to Liso et al. (1984). Two units of ascorbate oxidase (EC 1.10.3.3, Boehringer Mannheim) were used for each assay. Concentrations of AA were expressed as μg $g^{-1}$ wet weight of yeast cells and compared with a standard curve (1–5 μg of AA). The substantial stability (> 95%) of AA, during the utilized experimental methods of extraction and analysis, has been verified.

In order to exclude the ability of the selected yeast strains to utilize L-galactonic acid γ-lactone as a carbon source, all the strains were cultivated in YNB containing 0.5% L-galactonic acid γ-lactone, without any additional carbon source.

*Table 1.* Occurrence of ascorbate in five days cultures*

| Yeast strains | | Cells |
|---|---|---|
| | | μg $g^{-1}$ wet weight |
| *C. terreus* 6012 | C | n.d. |
| | T | n.d. |
| *C. terreus* 6242 | C | n.d. |
| | T | n.d. |
| *S. cerevisiae* 6174 | C | n.d. |
| | T | 8 |
| *S. cerevisiae* 6223 | C | n.d. |
| | T | 18 |
| *S. cerevisiae* 6248 | C | n.d. |
| | T | 17 |
| *C. lusitaniae* 6142 | C | n.d. |
| | T | 70 |
| *P. fermentans* 3808 | C | n.d. |
| | T | 71 |

C. Control culture. T. L-galactonic acid γ-lactone treated culture. n.d. not detectable. * All the cultures were in the stationary phase.

## Results

Within the tested yeast strains AA was not detected in control cultures, grown on a medium with glucose as sole carbon source. The addition of L-galactonic acid γ-lactone to the culture media revealed great differences in the AA production among the tested species and strains (Table 1). In *S. cerevisiae* (strains 6174, 6223 and 6248), *C. lusitaniae* 6142 and *P. fermentans* 3808, AA production was only detected by adding L-galactonic acid γ-lactone in the culture media. In the last two strains AA was detected in the cells reaching 70 and 71 μg $g^{-1}$ of wet weight, respectively.

In *C. terreus* 6012 and 6242 AA was not detected either in the presence of in the absence of L-galactonic acid γ-lactone.

All the strains tested were unable to grow on YNB containing L-galactonic acid γ-lactone, without glucose.

## Discussion

At present, biosynthesis and the role of AA in yeasts are still obscure. The last step in the pathway in baker's yeast (*S. cerevisiae*), is catalyzed by L-galactono γ-lactone oxidase, as reported by Nishikimi et al. (1978, 1980) and Bleeg and Christensen (1982), which isolated the enzyme from mitochondrial fraction.

Investigations here reported pointed out the existence of yeast strains such as *S. cerevisiae* (strains 6174, 6223 and 6248), *C. lusitaniae* 6142 and *P. fermentans* 3808, in which the addition of L-galactonic acid γ-lactone in the medium stimulates AA production. The amounts of AA obtained from cell homogenates are comparable to those reported for yeasts in similar growth conditions (Heick et al. 1972). These strains seem not able to produce AA when grown on a medium containing glucose as sole carbon source.

The fact that the addition of L-galactonic acid γ-lactone in the medium does not seem to influence the AA production in *C. terreus* 6012 and 6242, could suggest an alternative precursor for the AA biosynthesis in these strains. This hypothesis is strongly supported by experimental findings obtained with two filamentous fungi; in fact, Sivarama Sastry and Sarma (1957) reported the occurrence of AA in liquid media of *Aspergillus niger*, grown on sucrose as carbon source, as well as an enhanced production of the same metabolite in the presence of D-glucurono-γ-lactone. Similarly, Takahashi et al. (1960) detected D-araboascorbic acid in the cultural broths of different species of *Penicillium*, proposing D-glucono-γ-lactone as a possible intermediate.

Garuccio et al. (1989a, b), demonstrated that the growth of *C. terreus* 6012 and 6242 was not inhibited by lycorine, whereas all other strains here investigated were inhibited by lycorine in cell division (Garuccio & Arrigoni 1989).

The different responses to L-galactonic acid γ-lactone observed in *C. terreus* and in the other strains, here reported, are consistent with the differences they show in sensitivity to lycorine.

This alkaloid usually blocks cellular division in plants (De Leo et al. 1973; Liso et al. 1984), probably inhibiting the AA production (Arrigoni et al. 1975), i.e. the conversion of L-galactonic acid γ-lactone to ascorbate (De Gara et al. 1994). The fact that *C. terreus* 6012 and 6242 growth is not limited by lycorine is in accordance with the hypothesis that these strains could have a peculiar pathway for the AA biosynthesis. The additional suggestion that L-galactonic acid γ-lactone does not stimulate the AA production, being utilized as a carbon source, can not be sustained, since these strains are unable to grow on it, like the other ones here investigated.

On the basis of the above, it could be expected that yeast strains producing AA from L-galactonic acid γ-lactone, are inhibited by lycorine.

Investigation of the presence of enzymes producing AA in all the strains studied here, is in progress.

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